



Attorney Docket Number 54763-8001US02

## EXHIBIT B

PAGES FROM

LABORATORY NOTEBOOK

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Drug Sensitivity and growth curves

Cisplatin test.

6 wells per line x 2 → 12 wells for duplicates.

Concentrations:

- 0
- 0.1  $\mu M$
- 0.5 "
- 2.0 "
- 10.0 "
- 50.0 "

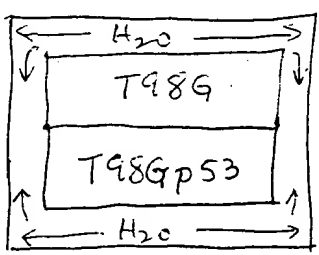


plate  $2 \times 10^4$  cells per well, settle overnight.

treat 1 hour w/ cisplatin = 1 hr. 15 min.

Count cells on

Growth curve

6 wells per line x 2 → 12 wells.

2000 cells per well.

T98Gp53 (culture previously assayed on ) kept without G418 since

1 almost confluent 6 cm dish trypsinized and resuspended in 5 ml.

$$\frac{103 \text{ cells}}{9 \text{ squares}} = 11 \times 10^4 \text{ per ml.}$$

$$\frac{11,000}{100 \mu l} = 110 \text{ cells} / 100 \mu l$$

$$\frac{22,000}{200 \mu l} \leftarrow \text{per well. 48 well dish.}$$

for drug test, add 200  $\mu l$  per well + 500  $\mu l$  medium.

for growth curve, plate 1500 cells per well.

$$\frac{11,000 \text{ cells}}{100 \mu l} = 110 \text{ cells} / 100 \mu l$$

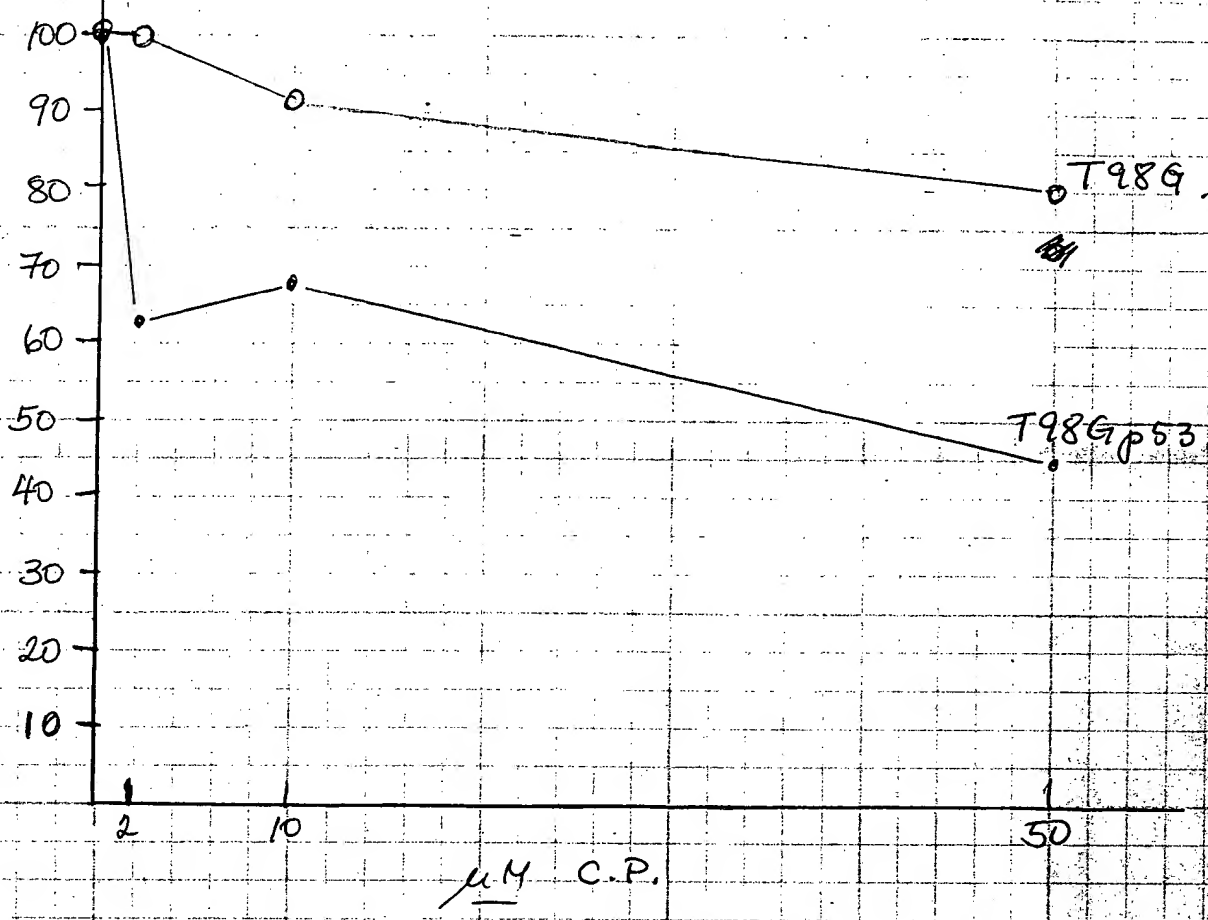
$$\frac{1500 \text{ cells}}{14 \mu l}$$

196  $\mu l$  (14 wells) into 6.8 ml. → 7 ml total, 0.5 ml per well.

42,381 30 SHEETS 3 SQUARE  
42,380 100 SHEETS 3 SQUARE  
42,380 200 SHEETS 3 SQUARE  
NATIONAL



# Cisplatin sensitivity



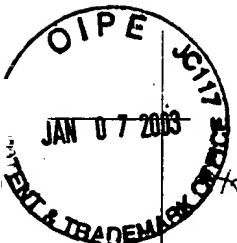
cells plated in 48 well plates, 2 wells per point, at 20,000 cells per well (very dense).

cells treated 1 hr w/ CP medium changed, and cell counts were made one week later.

plating  
treatment  
counts.

42,381 50 SHEETS 5 SQUARE  
42,382 100 SHEETS 5 SQUARE  
42,389 200 SHEETS 5 SQUARE  
MADE IN U.S.A.  
NATIONAL

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TECHNICAL DIVISION



Cisplatin test. T98G, T98G p53 (Growth w/o 5418)

Stock Cisplatin in DMEM at 4 mM.

treatment done in 48-well plates, 0.5 ml per well.  
170,000 cells per well plated out on previous day

prepare 2 mls each concentration:

10 $\mu$ M	add 5 $\mu$ l stock (5 $\mu$ l $\cdot$ 4 mM in 2 ml.)
20 $\mu$ M	10 $\mu$ l "
30	15 $\mu$ l.
40	20
60	30.
120	60
240	120.

following 1 hr. treatment, trypsin cells w/ 100  $\mu$ l trypsin, add 40  $\mu$ l complete medium; pipet up and down w/ 100  $\mu$ l tip to disperse, then replat in duplicate

5  $\mu$ l  $\rightarrow$  2 plates (~100 colonies)  
and/or 100  $\mu$ l  $\rightarrow$  2 plates (~2000 colonies).

as follows:

control	(0)	100 (x2 plates)	2000 (x2 plates)
10 $\mu$ M		"	"
20 $\mu$ M		"	"
30 $\mu$ M		"	"
40 $\mu$ M		—	"
60 $\mu$ M		—	"
120 $\mu$ M		—	"
240 $\mu$ M		—	"
		8 plates	16 plates.
24 plates per line.			

The remaining cells in 48 well dish were allowed to continue to grow and were counted on

42-381 100% RECYCLED PAPER 5 SQUARE  
42-382 100% RECYCLED PAPER 5 SQUARE  
42-383 100% RECYCLED PAPER 5 SQUARE  
42-384 100% RECYCLED PAPER 5 SQUARE  
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42-398 100% RECYCLED PAPER 5 SQUARE  
42-399 100% RECYCLED PAPER 5 SQUARE  
42-400 100% RECYCLED PAPER 5 SQUARE  
Made in U.S.A.



1484 p<sup>3</sup>

10  $\mu$ M 68 x 4 x 10<sup>4</sup> / ml.

60  
48  
66  
(118)  
242

20  $\mu$ M 53  
56 (139)  
64

30  $\mu$ M 24  
22 (96)  
26

40  $\mu$ M  
( $\frac{16}{3}$ ) x 4 x 10<sup>4</sup>  
( $\frac{17}{3}$ ) (25)  
( $\frac{23}{3}$ )

60  $\mu$ M  $\frac{3}{9}$  x 4 x 10<sup>4</sup> / ml. (1.3)

120  $\mu$ M ( $\frac{1}{9}$ ) (0.4)

240  $\mu$ M  $\frac{1}{9}$  (0.4)

0 41

% max # cells.

10  $\mu$ M 100%  
20  $\mu$ M 95%  
30  $\mu$ M 40%  
40  $\mu$ M 10%  
60  $\mu$ M 0.5%  
120  $\mu$ M 0.17%  
240  $\mu$ M "

CELL CENTER 1800500

7989C = 2

~~88~~ x 4 x 10<sup>4</sup> / ml.

~~83~~  
97  
110  
104  
(~~383~~)  
(415) 100%

40  $\mu$ g

85  
82  
96  
(364) 80%

10  $\mu$ g

115  
110  
97  
(429) 103

60  $\mu$ g

23  
19  
17  
25  
84 20%

20  $\mu$ g

~~85~~  
~~77~~  
104  
106  
84  
(365) 88  
379 91

120  $\mu$ g

23  
9  
10 2.4%

30  $\mu$ g

95  
85  
~~77~~  
97  
(407) 98  
89

240  $\mu$ g

4  
9  
1.8 0.4%

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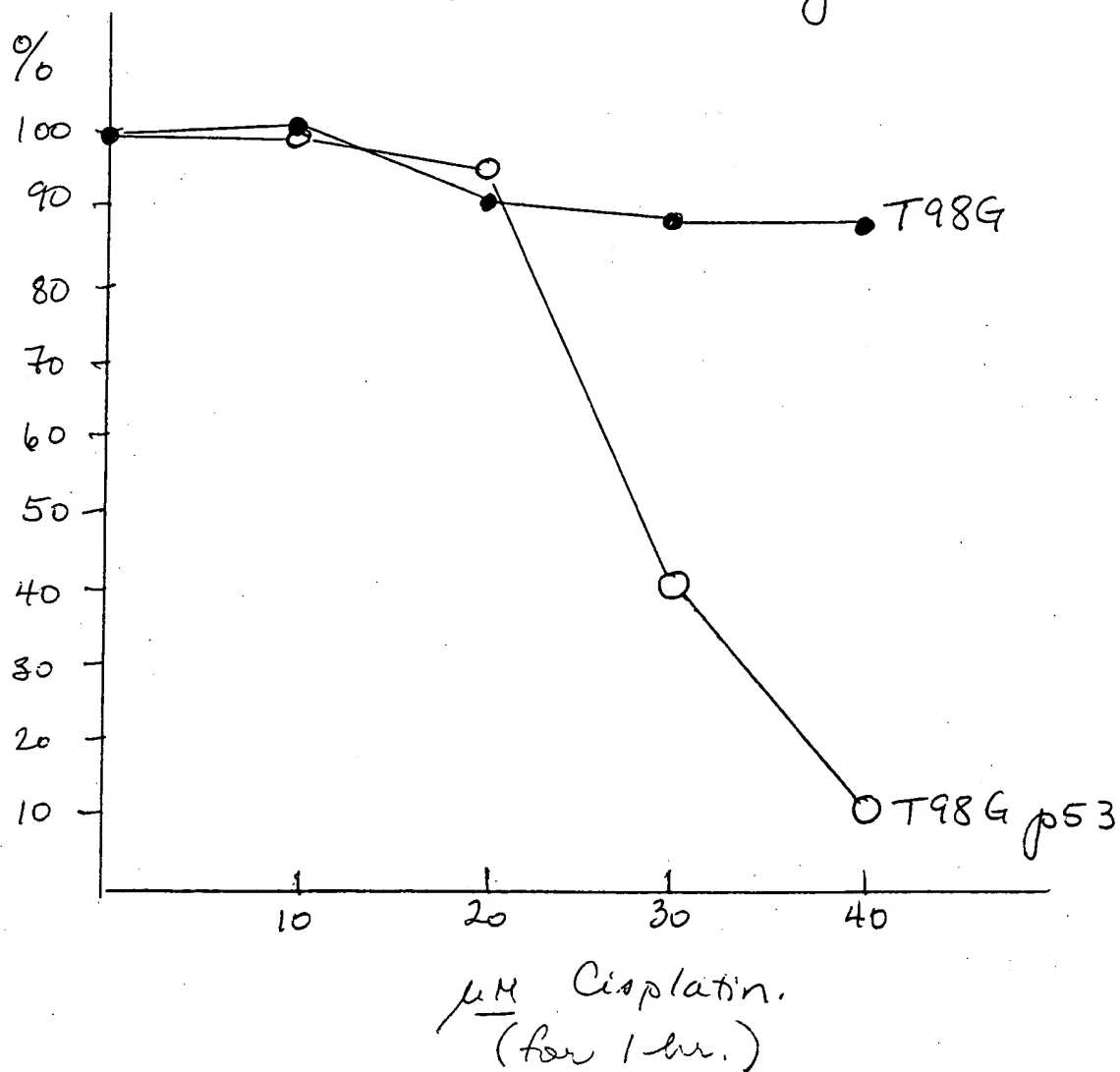


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42-598 10531229166855718669791802768367043231889509540054911125431097753600 SHEETS 5 SQUARE  
42-599 21062458333711437339583605536734086463779019080109822250862195507200 SHEETS 5 SQUARE  
42-600 42124916667422874679167211073468172927558038160219644501724391014400 SHEETS 5 SQUARE  
42-601 84249833334845749358334422146936345855116076320439289003448782028800 SHEETS 5 SQUARE  
42-602 168499666669691498716668844293872691710232152640878578006897564057600 SHEETS 5 SQUARE  
42-603 336999333339382997433337688587745383420464305281757156013795128115200 SHEETS 5 SQUARE  
42-604 673998666678765994866675377175490766840928610563514312027590256230400 SHEETS 5 SQUARE  
42-605 1347997333357531989733350754350981533681857221127028624055180512460800 SHEETS 5 SQUARE  
42-606 2695994666715063979466701508701963067363714442254057248110361024921600 SHEETS 5 SQUARE  
42-607 5391989333430127958933403017403926134727428884508114496220722049843200 SHEETS 5 SQUARE  
42-608 10783978666860255917866806034807852269454857769016228992441444099686400 SHEETS 5 SQUARE  
42-609 21567957333720511835733612069615704538909715538032457984882888199372800 SHEETS 5 SQUARE  
42-610 43135914667441023671467224139231409077819431076064915969765776398745600 SHEETS 5 SQUARE  
42-611 86271829334882047342934448278462818155638862152129831939531552797491200 SHEETS 5 SQUARE  
42-612 172543658669764094685868896556925636311277724304259663879063105594982400 SHEETS 5 SQUARE  
42-613 345087317339528189371737793113851272622555448608519327758126211189964800 SHEETS 5 SQUARE  
42-614 690174634679056378743475586227702545245110897217038655516252422379929600 SHEETS 5 SQUARE  
42-615 1380349269358112757486951172455405090490221794434077311032504844759859200 SHEETS 5 SQUARE  
42-616 2760698538716225514973902344910810180980443588868154622065009689519718400 SHEETS 5 SQUARE  
42-617 5521397077432451029947804689821620361960887177736309244130019379039436800 SHEETS 5 SQUARE  
42-618 11042794154864902059895609379643240



Cisplatin sensitivity of T98G cells.  
T98Gp53

% maximum cell growth at 7 days



42.381 50 SHEETS 5 SQUARE  
42.382 100 SHEETS 5 SQUARE  
42.389 200 SHEETS 5 SQUARE



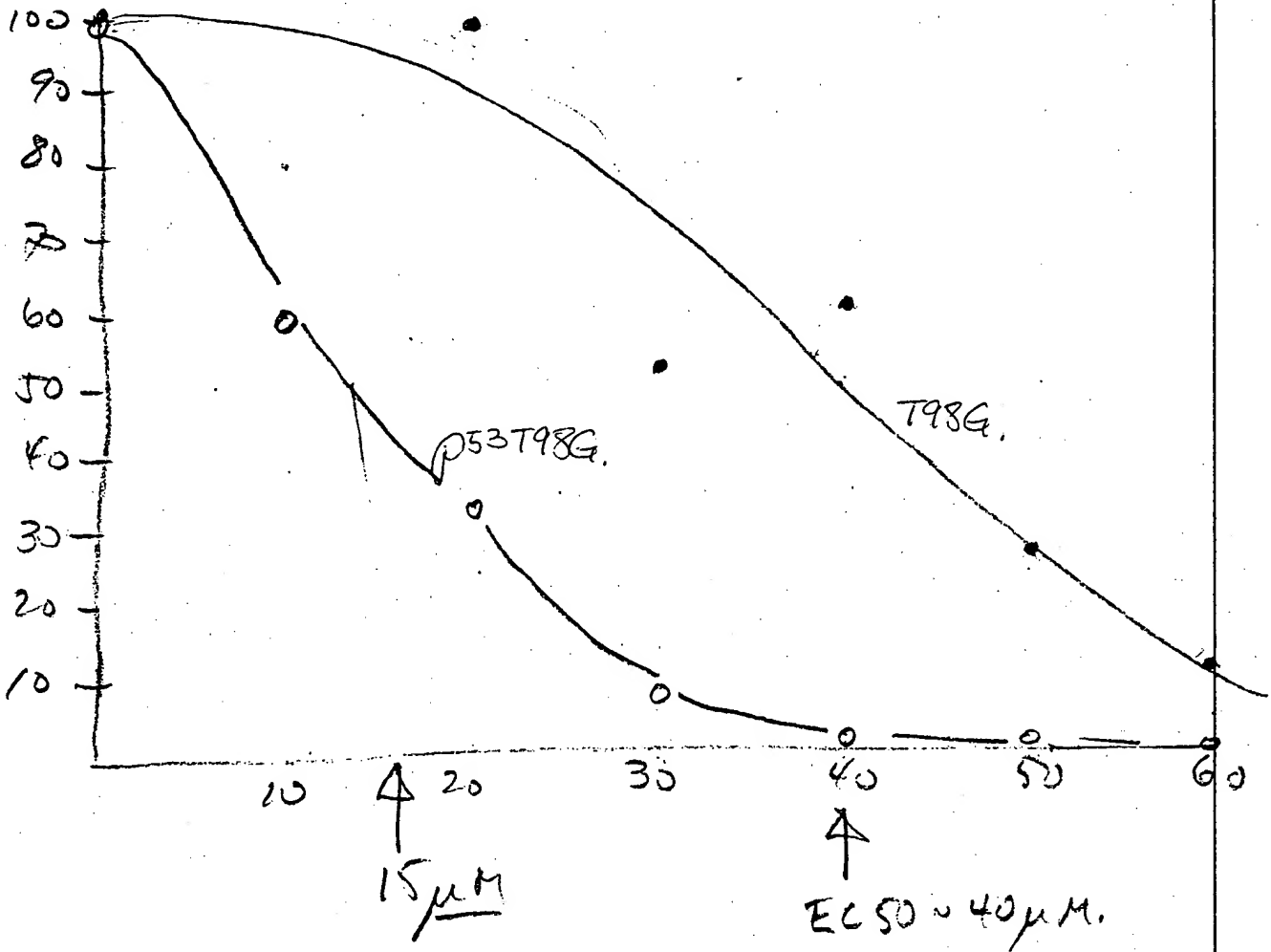
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RECEIVED





Taxon's Repeat of the Cisplatin  
Sensitivity experiment.

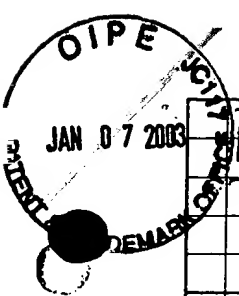


42-381 50 SHEETS EYE-EASE® 5 SQUARE  
42-382 100 SHEETS EYE-EASE® 5 SQUARE  
42-383 200 SHEETS EYE-EASE® 5 SQUARE  
42-384 100 SHEETS EYE-EASE® 10 SQUARE  
42-385 200 SHEETS EYE-EASE® 10 SQUARE  
42-386 200 RECYCLED WHITE 5 SQUARE  
Made in U.S.A.

National Brand



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FOR OFFICIAL USE ONLY



153  
1986

1986

0 A

$\frac{107}{9}$  }  $12.3 \times 10^4$   
 $\frac{115}{9}$  }

11.5  
100%

$\frac{50}{55}$  } 53.5  
 $\frac{44}{45}$  }

54

0 B

$\frac{93}{9}$  }  $10.7 \times 10^4$   
 $\frac{99}{9}$  }

$\frac{52}{46}$  } 54  
 $\frac{46}{46}$  }

49.3

10 C

$\frac{63}{9}$  } 6.7 58%  
 $\frac{57}{9}$  }

$\frac{37}{9}$  } 42

78%  
85%

20 D

$\frac{42}{9}$  } 4.3 37  
 $\frac{36}{9}$  }

$\frac{55}{63}$  } 60  
 $\frac{46}{56}$  }

55  
56 51.3 100%  
43 110  
49

30 E

$\frac{9}{9}$  } 1.0 9  
 $\frac{46}{9}$  }

$\frac{26}{34}$  } 29.2  
 $\frac{32}{32}$  }

32 54%  
23.2 5  
19.1  
30

40 F

$\frac{1}{9}$  } 0.11 1  
 $\frac{1}{9}$  }

$\frac{36}{31}$  } 34  
 $\frac{35}{35}$  }

33

61%  
67

50 G

$\frac{1}{9}$  } 0.4 1

$\frac{18}{20}$  } 17.5  
 $\frac{20}{12}$  }

18

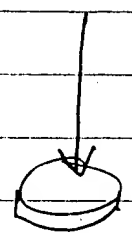
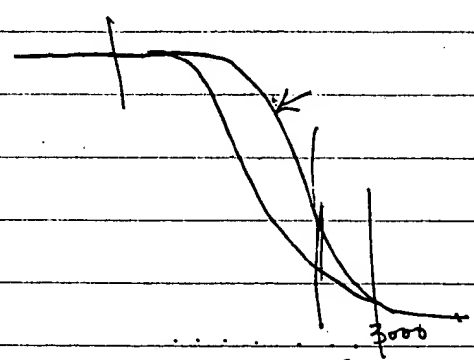
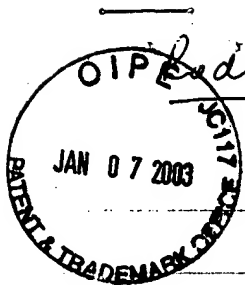
83%  
35

$\frac{1}{9}$  } 0.05 0.5%  
 $\frac{1}{9}$  }

$\frac{4}{16}$  } 13.5  
 $\frac{14}{10}$  }

14

26%  
27



50"  
1000 rads ~~trans.~~  
500 rads 25"  
60 rads ~~3"~~  
3"

1000  
5 x 10<sup>5</sup>  
20 3000

2.3 x 10<sup>5</sup> cells  
10<sup>6</sup> cells  
5.10<sup>5</sup> / T25  
5 x 10<sup>4</sup> / T25

20 0.5 · 10<sup>3</sup>  
4 · 10<sup>4</sup>

60	<del>60</del>	3"
80	100	5"
100	200	10"
140	300	15"
180	400	20"
220	500	25"
260	600	30"
300	700	35"
340	800	40"
	900	45"
	1000	50"
	1500	75"

1500 ~ 0.3 x 10<sup>6</sup>  
4 · 10<sup>4</sup>

15 · 10<sup>5</sup> ~ 0.3 · 10<sup>6</sup>  
40 · 10<sup>3</sup>

T25 25  
50,000 T25  
10,000

12 flasks each line.

5000 cells per T25

2000  
T98G

3 series of 12 T25 flasks were set up on

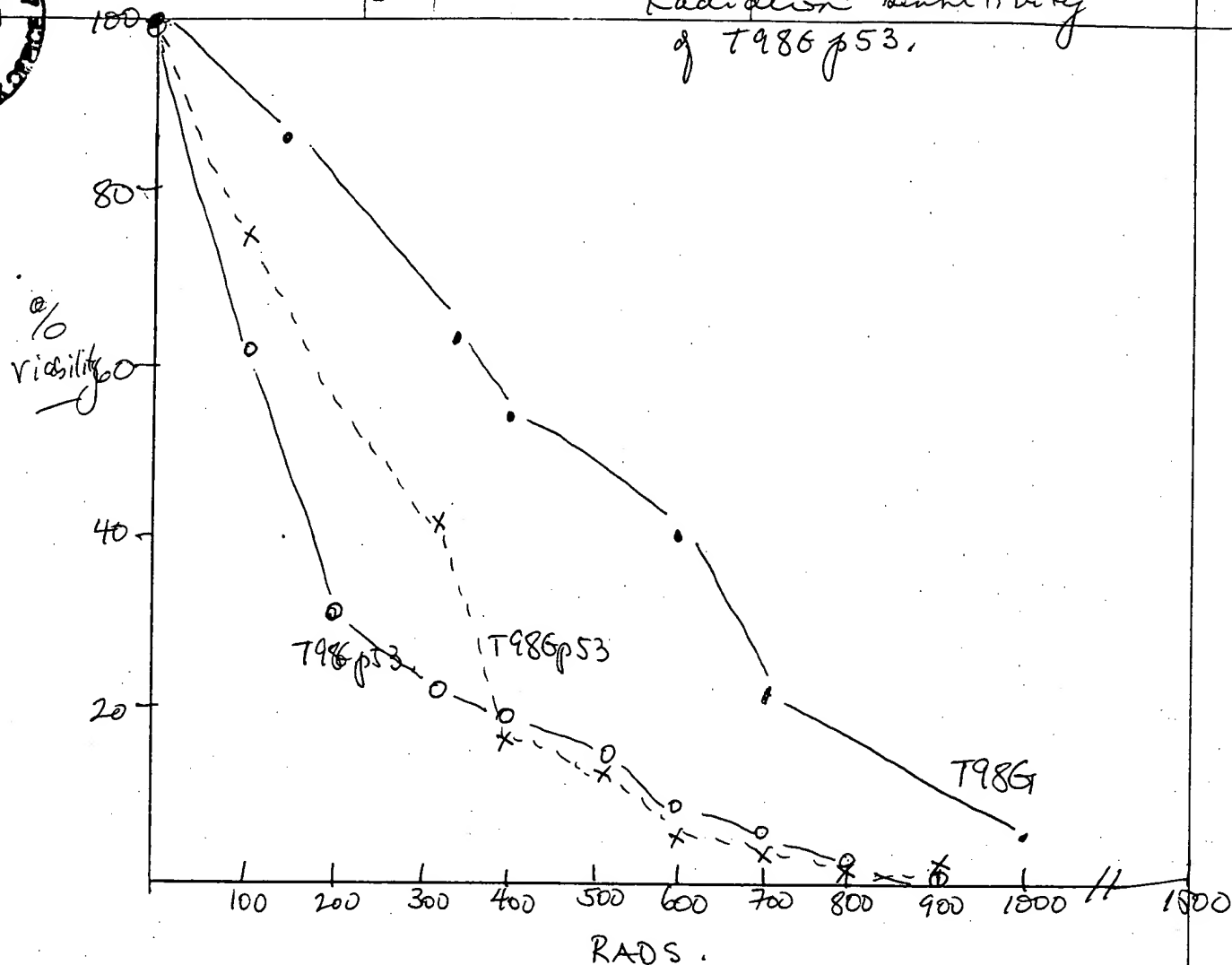
- ① T98G parental line
- ② T98G p53 propagated in G418 until time of plating
- ③ T98G p53 propagated two weeks w/o G418.

Cells were allowed to attach for 24 hrs prior to irradiation.

[illegible]



Radiation sensitivity  
of T986p53.



Colonies of T986 counted at 5 days.

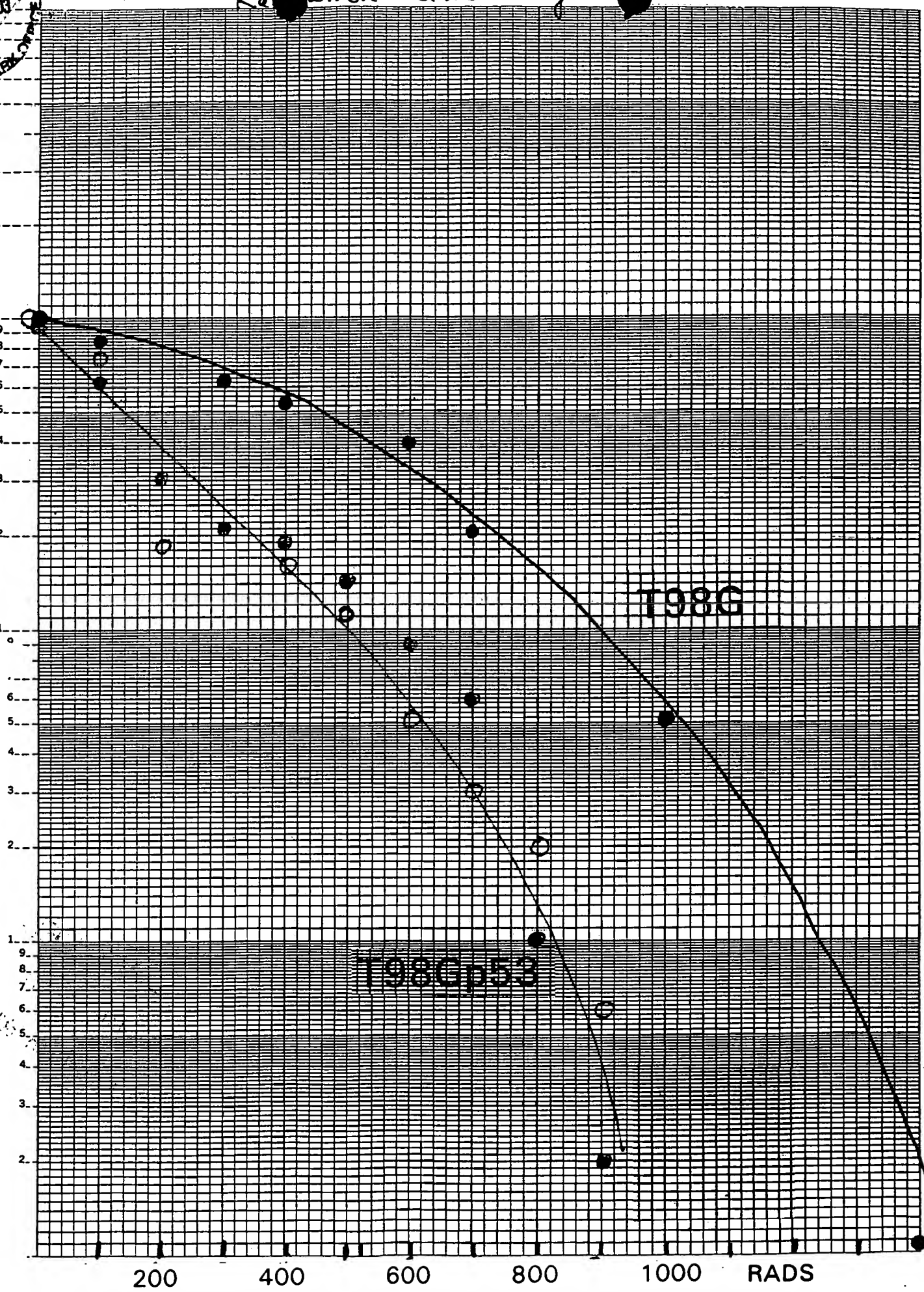
Colonies of T986p53 counted at 12 days.

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TECH CENTER

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PATENT & TRADEMARK OFFICE

# Radiation Sensitivity

PERCENT SURVIVAL



T98G

T98Gp53